PATENT
DOCKET NO. P50444-9D2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

NEW APPLICATION TRANSMITTAL LETTER UNDER 37 CFR § 1.53(b)(1)

Sir:

Transmitted herewith is the patent application of the below named inventor(s), pursuant to 37 CFR § 1.53(b)(1). Applicants request that the application be filed as a Divisional Application of U.S. Serial No. 08/978,456, filed November 25, 1997.

Inventors: MICHAEL TERENCE BLACK, a citizen of the United Kingdom; MARTIN KARL RUSSELL BURNHAM, a citizen of the United Kingdom; JASON CRAIG FEDON, a citizen of the United States of America; JOHN EDWARD HODGSON, a citizen of the United Kingdom, DAVID JUSTIN CHARLES KNOWLES, a citizen of the United Kingdom, MICHAEL ARTHUR LONETTO, a citizen of the United States of America, RICHARD OAKLEY NICHOLAS, a citizen of the United Kingdom; LESLIE MARIE PALMER, a citizen of the United States of America; JULIE M. PRATT, a citizen of the United Kingdom; RAYMOND WINFIELD REICHARD, a citizen of the United States of America; MARTIN ROSENBERG, a citizen of the United States of America; CHRISTOPHER MICHAEL TRAINI, a citizen of the United States of America; JUDITH M. WARD, a citizen of the United Kingdom; RICHARD LLOYD WARREN, a citizen of the United States of America.

Title: ribG

1. Papers enclosed which are required for filing date Under 37 CFR § 1.53(b):

1	Title Page (Page In Front of Specification)
33	page(s) of specification (minus claims and abstract)
5	page(s) of claims
1	page(s) of abstract
8	page(s) of sequence listing
0	sheet(s) of drawing
10	page(s) of declaration and power of attorney **(original or copy)**
0	page(s) Preliminary Amendment

"Express Mail" mailing label number EJ 691 698 603 US

I hereby certify that this paper and the documents referred to as attachments therein are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" under 37 CFR 1.10 on **August 18, 1999**, and addressed to: Assistant Commissioner for Patents, Washington, DC 20231.

Heather S. Richman
Heather Richman

2. Additional Papers enclosed:

☒ Acknowledgment postcard

☒ Associate Power of Attorney

3. Oath or Declaration

(a) ☐ Newly executed (original or copy)

(b) ☒ Copy from a prior application (37 CFR § 1.63(d)(1)(iv)) for Divisional Application

(c) ☐ Declaration or oath is not enclosed. **[OPTIONAL]**

(i) ☐ Deletion of Inventor(s)

Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR §§ 1.63(d)(2) and 1.33(b).

4. ☒ **Incorporation By Reference.**
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied and is considered to be part of the disclosure of the accompanying application and is hereby incorporated by reference herein.

5. **If a Continuing Application**, *check appropriate box, and supply the requisite information below and in a preliminary amendment*:

☐ Continuation ☒ Divisional ☐ Continuation-in-part (CIP)

of prior Application No. 08/978,456

Prior application Information: Examiner: Navarro

Group/Art Unit: 1645

6. **Co-Pendency**

☒ No extension of time is believed needed to maintain the co-pendency of the parent application.

☐ An extension of time in the parent case is filed herewith.

Should an extension of time or an additional extension of time in the parent case be required to maintain co-pendency, please consider this a Petition for such extension. Any additional fees required for such extension may be charged to Deposit Account No. 50-0258.

7. The correspondence address

☐ remains the same as in the prior Application

☒ should be revised to recite:

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8. **Claim of Priority to Foreign Application:** Priority under 35 U.S.C. § 119 is claimed based on **[TITLE] ["   "]** based on U.S. Application No. ____________________, filed _________________.

9. This application claims priority of one or more U.S. Applications under 35 U.S.C. §120. Accordingly, please amend the application to recite as the first line of the application:

   --This application is a divisional of U.S. Application No. 08/978,456 filed November 25, 1997.--

10. An assignment of the invention of the application, together with an Assignment Recordation Cover Sheet:

   ☐ is enclosed, or

   ☒ was filed in a parent case and recorded at

   Reel No.: Not yet known
   Frame No.: Not yet known

11. ☐ Microfiche Computer Program (Appendix)

12. Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary)

   A. Enclosed are:

   (a) ☐ Computer Readable Copy of the Sequence Listing

   (b) ☐ Paper Copy (identical to Computer Readable Copy) of the Sequence Listing

   B. ☒ Enclosed is a paper copy of the Sequence Listing. This paper copy and a Computer Readable Form thereof are identical with the Computer Readable Form in another application of the Applicant which is fully identified as follows:

   U.S. Application No.08/978,456

   Filed: November 25, 1997

   Attorney Docket No.: P50444-09

which is believed to comply with the rules set forth in 37 CFR § 1.821 et. seq. Applicants requests pursuant to 37 CFR § 1.821(e) that this Computer Readable Form be used in the present application.

☒ Statement under 37 CFR § 1.821(f): The content of the Computer Readable Copy enclosed or identified above as in another application of the Applicant is the same as that of the paper copy.

13. Prior to the examination of this Application, please:

☐ enter the enclosed Preliminary Amendment

14. **Information Disclosure Statement:**

- The Examiner is requested to consider carefully the complete text of the documents submitted herewith in connection with the examination of this application. It is believed that the Examiner will concur with Applicant's belief that the documents do not adversely affect the patentability of the subject matter presently claimed, taken alone or in combination.
- It is requested that the listed documents be included in the "References Cited" portion of any patent issuing from this application.
- Under 37 CFR 1.97(i), Applicants understand that non-complying Information Disclosure Statements will be placed in the file but not considered by the Office, however, under Reply to Comment 8 of the Federal Register, page 2024, Applicants will be informed when information is not considered.

[X] Applicants make of record the documents submitted in parent Application Serial No. 08/978,456, filed November 25, 1997. These documents are listed on the Form

[ ] PTO/SB/08A
[ ] PTO/SB/08B
[X] PTO-1449

which was submitted in the parent case, a copy of which form(s), modified to recite the new filing information, is/are enclosed. The Office is requested to make these forms of record in the present case. In accordance with 37 CFR 1.98(d), copies of the documents cited in the above-listed forms are not enclosed.

[ ] If additional documents are to be made of record, these are listed with unique identifiers on further Forms PTO/SB/08A and PTO/SB/08B which are enclosed. Copies of these additional documents are enclosed.

15. Fee Calculation for filing of Application, taking into account the above-referenced amendments:

☒ Other Than Small Entity ($760.00)

☐ Small Entity ($380.00)

☐ Claims in Excess of 20: (@ $18.00 Other Than Small Entity)

☐ Claims in Excess of 20: (@ $9.00 Small Entity)

☒ Independent Claims in Excess of 3: (2 @ $78.00 Other Than Small Entity)

☐ Independent Claims in Excess of 3: (@ $39.00 Small Entity)

☐ First Presentation of Multiple Dependent Claim <u>($260.00 Other Than Small Entity or $130.00 Small Entity)</u>

Fee payment being made at this time is enclosed:

*	Basic filing fee **(Small/Other Than Small Entity)**	$760.00
*	Claims in Excess of 20: (@ $18.00 Other Than Small Entity)	*.00
*	Claims in Excess of 20: (@ $9.00 Small Entity)	*.00
*	Independent Claims in Excess of 3: (2 @ $78.00 Other Than Small Entity)	156.00
*	Independent Claims in Excess of 3: (@ $39.00 Small Entity)	*.00
*	Total Fees enclosed:	<u>$916.00</u>

16. The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 50-0258. This letter is filed in duplicate for accounting purposes.

Respectfully submitted,

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Docket No. P50444-09

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Title: ribG

ribG

RELATED APPLICATIONS

This application claims benefit of PCT Application, Number PCT/US97/02318, filed February 19, 1997.

FIELD OF THE INVENTION

This invention relates to newly identified polynucleotides and polypeptides, and their production and uses, as well as their variants, agonists and antagonists, and their uses. In particular, the invention relates to novel polynucleotides and polypeptides of the riboflavin specific deaminase family, hereinafter referred to as "ribG".

BACKGROUND OF THE INVENTION

It is particularly preferred to employ Staphylococcal genes and gene products as targets for the development of antibiotics. The Staphylococci make up a medically important genera of microbes. They are known to produce two types of disease, invasive and toxigenic. Invasive infections are characterized generally by abscess formation effecting both skin surfaces and deep tissues. S. aureus is the second leading cause of bacteremia in cancer patients. Osteomyelitis, septic arthritis, septic thrombophlebitis and acute bacterial endocarditis are also relatively common. There are at least three clinical conditions resulting from the toxigenic properties of Staphylococci. The manifestation of these diseases result from the actions of exotoxins as opposed to tissue invasion and bacteremia. These conditions include: Staphylococcal food poisoning, scalded skin syndrome and toxic shock syndrome.

Riboflavin (vitamin B2) is a member of the B complex of vitamins which function as coenzymes in metabolic reactions. Riboflavin has two coenzyme forms, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) which act in oxidation-reduction reactions such as the cytochrome system of electron transport and the oxidative degradation of pyruvate, fatty acids and amino acids. The first committed step in the biosynthesis of riboflavin is the opening

of the imidazole ring of GTP. In the presence of 3 H_2O and Mg^{++}, the C-8 of GTP is released as formate accompanied by the release of pyrophosphate by the action of GTP cyclohyrolase II (GCH2; EC 3.5.4.25). This enzyme function is encoded by *ribA* in bacteria and *rib1* in yeast species. Through a series of steps, involving 3,4-dihydroxy-2-butanone 4 phosphate synthase (*ribA*), 6,7-dimethyl-8-ribityllumazine synthase (*ribH*), riboflavin synthase (*ribB*), pyrimidine deaminase and pyrimidine reductase (*ribG*), enzymes encoded by genes within the riboflavin biosynthesis operon, riboflavin is formed. Because the genes required for riboflavin biosynthesis are present in many pathogenic microorganisms, and since riboflavin biosynthesis has shown to be required for virulence in the swine pathogen *Actinobacillus pleuropneumoniae* (Fuller, TE, *et al.* (1996) A riboflavin auxotroph of *Actinobacillus pleuropneumoniae* is attenuated in swine. Infect. Immun. 64:4659-4664), these gen e products represent broad spectrum antibacterial as well as antifungal targets.

The frequency of *Staphylococcus aureus* infections has risen dramatically in the past few decades. This has been attributed to the emergence of multiply antibiotic resistant strains and an increasing population of people with weakened immune systems. It is no longer uncommon to isolate *Staphylococcus aureus* strains which are resistant to some or all of the standard antibiotics. This phenomenon has created a demand for both new anti-microbial agents, vaccines, and diagnostic tests for this organism.

Clearly, there exists a need for factors, such as the ribG embodiments of the invention, that have a present benefit of being useful to screen compounds for antibiotic activity. Such factors are also useful to determine their role in pathogenesis of infection, dysfunction and disease. There is also a need for identification and characterization of such factors and their antagonists and agonists to find ways to prevent, ameliorate or correct such infection, dysfunction and disease.

Certain of the polypeptides of the invention possess amino acid sequence homology to a known Bacillus subtilis ribG protein.

SUMMARY OF THE INVENTION

It is an object of the invention to provide polypeptides that have been identified as novel ribG polypeptides by homology between the amino acid sequence set out in Table 1 [SEQ ID NO:

2 or 4] and a known amino acid sequence or sequences of other proteins such as Bacillus subtilis ribG protein.

It is a further object of the invention to provide polynucleotides that encode ribG polypeptides, particularly polynucleotides that encode the polypeptide herein designated ribG.

In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding ribG polypeptides comprising a sequence set out in Table 1 [SEQ ID NO:1 or 3] which includes a full length gene, or a variant thereof.

In another particularly preferred embodiment of the invention there is a novel ribG protein from *Staphylococcus aureus* comprising the amino acid sequence of Table 1 [SEQ ID NO:2 or 4], or a variant thereof.

In accordance with another aspect of the invention there is provided an isolated nucleic acid molecule encoding a mature polypeptide expressible by the Staphylococcus aureus WCUH29 on deposit strain, which nucleic acid is contained in the deposited strain.

A further aspect of the invention there are provided isolated nucleic acid molecules encoding ribG, particularly *Staphylococcus aureus* ribG, including mRNAs, cDNAs, genomic DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and compositions comprising the same.

In accordance with another aspect of the invention, there is provided the use of a polynucleotide of the invention for therapeutic or prophylactic purposes, in particular genetic immunization. Among the particularly preferred embodiments of the invention are naturally occurring allelic variants of ribG and polypeptides encoded thereby.

Another aspect of the invention there are provided novel polypeptides of *Staphylococcus aureus* referred to herein as ribG as well as biologically, diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and compositions comprising the same.

Among the particularly preferred embodiments of the invention are variants of ribG polypeptide encoded by naturally occurring alleles of the ribG gene.

In a preferred embodiment of the invention there are provided methods for producing the aforementioned ribG polypeptides.

In accordance with yet another aspect of the invention, there are provided inhibitors to such polypeptides, useful as antibacterial agents, including, for example, antibodies.

In accordance with certain preferred embodiments of the invention, there are provided products, compositions and methods for assessing ribG expression, treating disease, assaying genetic variation, and administering a ribG polypeptide or polynucleotide to an organism to raise an immunological response against a bacteria, especially a *Staphylococcus aureus* bacteria.

In accordance with certain preferred embodiments of this and other aspects of the invention there are provided polynucleotides that hybridize to ribG polynucleotide sequences, particularly under stringent conditions.

In certain preferred embodiments of the invention there are provided antibodies against ribG polypeptides.

In other embodiments of the invention there are provided methods for identifying compounds which bind to or otherwise interact with and inhibit or activate an activity of a polypeptide or polynucleotide of the invention comprising: contacting a polypeptide or polynucleotide of the invention with a compound to be screened under conditions to permit binding to or other interaction between the compound and the polypeptide or polynucleotide to assess the binding to or other interaction with the compound, such binding or interaction being associated with a second component capable of providing a detectable signal in response to the binding or interaction of the polypeptide or polynucleotide with the compound; and determining whether the compound binds to or otherwise interacts with and activates or inhibits an activity of the polypeptide or polynucleotide by detecting the presence or absence of a signal generated from the binding or interaction of the compound with the polypeptide or polynucleotide.

In accordance with yet another aspect of the invention, there are provided ribG agonists and antagonists, preferably bacteriostatic or bacteriocidal agonists and antagonists.

In a further aspect of the invention there are provided compositions comprising a ribG polynucleotide or a ribG polypeptide for administration to a cell or to a multicellular organism.

Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following descriptions and from reading the other parts of the present disclosure.

DESCRIPTION OF THE INVENTION

The invention relates to novel ribG polypeptides and polynucleotides as described in greater detail below. In particular, the invention relates to polypeptides and polynucleotides of a novel ribG of *Staphylococcus aureus*, which is related by amino acid sequence homology to Bacillus subtilis ribG polypeptide. The invention relates especially to ribG having the nucleotide and amino acid sequences set out in Table 1 as SEQ ID NO: 1 and SEQ ID NO: 2 respectivelyribG.

TABLE 1

ribG Polynucleotide and Polypeptide Sequences

(A) Sequences from *Staphylococcus aureus* ribG polynucleotide sequence [SEQ ID NO:1].

```
5'-
ATGGATTATGCGATTCAACTTGCAAATATGGTACAAGGTCAAACAGGTGTTAATCCACCCGTTGGCGCTG
TTGTAGTTAATGAAGGTAGGATTGTTGGTATTGGTGCACACTTGAGAAAAGGTGACAAGCATGCGGAGGT
TCAAGCACTTGATATGGCACAACAAAATGCTGAAGGTGCGACGATTTATATTACGTTAGAGCCATGTAGT
CATTTTGGTTCAACACCACCCTGTGTTAACAAAATTATTGATTGTAAGATAGCAAAAGTAGTATACGCAA
CAAAAGACAATTCGTTAGACACACATGGTGATGAGACGTTACGGGCTCACGGTATTGAGGTTGAATGCGT
TGATGATGAACGGGCATCACAATTATACCAAGACTTTTTTAAAGCAAAAGCAAAGCAACTGCCACAAATT
ACAGTGAAAGTATCTGCAAGTTTAGATGGTAAACAAGCGAATGATAATGGACAAAGTCAATGGATTACTA
ACAAAGAGGTTAAACAAGATGTCTATAAGTTAAGACATCGACACGACGCAGTGTTAACTGGAAGACGTAC
AGTTGAATTAGATGATCCACAATATACTACACGTATTCAAGATGGAAAAAACCCTATAAAAGTAATATTG
TCTAAGTCTGGGAATATTCATTTTAATCAGCAAATTTATCAAGATGAATCAACACCAATTTGGATATATA
CTGAAAATCCAAATTTAACAAGCAATCAAACACATATTGAAATTATTTACTTGAAGTCTTGTGATTTAAC
AACAATTCTTCACAATTTATATAAAAGAGGAGTTGGAACTTTGCTAGTCGAGGCAGGTCCAACCACTACT
TCAGAATTCTCCATCTATTATATAGATGAATTTATTCTCTATTATGCCCCGAAATTAATTGGCGGATCTG
GAAATTATCAATTTTATCAAACAAATGATGTGATTGAGATACCAGATGCGAACCAATTTGAAATTGTTCA
TTCCGAGTTATTAAATCAAAATGTTAAATTAACTTTACGAAAGAAGTGA-3'
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(B) *Staphylococcus aureus* ribG polypeptide sequence deduced from the polynucleotide sequence in this table [SEQ ID NO:2].

NH_2-
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MDYAIQLANMVQGQTGVNPPVGAVVVNEGRIVGIGAHLRKGDKHAEVQALDMAQQNAEGATIYITLEPCS
HFGSTPPCVNKIIDCKIAKVVYATKDNSLDTHGDETLRAHGIEVECVDDERASQLYQDFFKAKAKQLPQI
TVKVSASLDGKQANDNGQSQWITNKEVKQDVYKLRHRHDAVLTGRRTVELDDPQYTTRIQDGKNPIKVIL
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SKSGNIHFNQQIYQDESTPIWIYTENPNLTSNQTHIEIIYLKSCDLTTILHNLYKRGVGTLLVEAGPTTT
SEFSIYYIDEFILYYAPKLIGGSGNYQFYQTNDVIEIPDANQFEIVHSELLNQNVKLTLRKK-COOH

(C) Polynucleotide sequences comprising *Staphylococcus aureus* ribG ORF sequence [SEQ ID NO:3].

5'-
AANCACCAATCCNATTGGGAGGNAATCCAAATCAATNCCCGGANNCCCAATCCAAGTTAATTAAGTCCAA
GGTTTTGGAACATTACCAAATATGATTCCGATGAGGTCAAATGNCAANCGGTGTTAATAAACTACGAAAT
GNTGTGNAAATGATAGTAGANCAAGTTGCGCATACAGTNTCTCNATTATATGATGCTTTAGAATCGAATG
AGCAACAACAGCGCAGTTACAATCAATAATTTGTAACTAGAAGATAATAAAGAGAACGCTCTATAGAGAC
GAATTGAAGGTTTGATTTTAATGTCTGTTAGTAAGAATCATATCAATGAGATGCCTATAGTACTCAGATT
ATATTAAATTAAAACCGTCATTAATTGTTTTTTTAGAAAACATATAGTATCATTTTAAATGTAGTTGACA
TACTACGTACTCAAATAATCTATAACAATTTCATATATAATTCTTTCGGGGCAGGGTGAAATTCCCAACC
GGCAGTAAATAAAGCCTGCGACCTGCTAATATGTTTCATATTAGTGGCTGATCTAGTGAGATTCTAGAGC
CGACAGTTAAAGTCTGGATGGGAGAAAGAATGTTAATTATCGACAAAGATAATGTAGCGTATTTGTAAAA
ATGTGTACAAATAGGCTTATTTAACGATAA
ATTTTTCTCCTTTGCATCTTAATTCATGATGTGAGGATTTTTTGTTTATAGAGGTGATCATTTGAGTCAA
TTTATGGATTATGCGATTCAACTTCCAAATATGGTACAAGGTCANACAGGTGTTAATCCACCCGTTGGCG
CTGTTGTAGTTAATGAAGGTAGGATTGTTGGTATTGGTGCACACTTGAGAAAAGGTGACAAGCATGCGGA
GGTTCAAGCACTTGATATGGCACAACANAATGCTGAAGGTGCGACGATTTATATTACGTTAGAGCCATGT
AGTCATTTTGGTTCAACACCACCCTGTGTTAACAAAATTATTGATTGTAAGATAGCANAAGTAGTATTAC
NCAACANAAGACAATTCCGTTAGACACACATGGGTGATGAGACGTTACGGGGCTCCACGGTATTTGAGGG
TTGAATTGCGTTGGATGATGAACGGGCATCACAATTATACCAAAGACTTTTTTTAAAGCAAAAAGCAAAG
CAACTTGCCACAAAATTACAGTGAAAGTNTCTTGAAAGTTTAGATGGGTAAACAAAGCGAATTGATAATG
GACAAAGTCAATGGATTACTAACAAAGAGGTTAAACAAGATGTCTATAG-3'

(D) *Staphylococcus aureus* ribG polypeptide sequence deduced from the polynucleotide ORF sequence in this table [SEQ ID NO:4].

NH_2-
MDYAIQLPNMVQGXTGVNPPVGAVVVNEGRIVGIGAHLRKGDKHAEVQALDMAQXNAEGATIYITLEPCS
HFGSTPPCVNKIIDCKIAXVVLXNXRQFR -COOH

Deposited materials

A deposit containing a *Staphylococcus aureus* WCUH 29 strain has been deposited with the National Collections of Industrial and Marine Bacteria Ltd. (herein "NCIMB"), 23 St. Machar Drive, Aberdeen AB2 1RY, Scotland on 11 September 1995 and assigned NCIMB Deposit No.

40771, and referred to as Staphylococcus aureus WCUH29 on deposit. The *Staphylococcus aureus* strain deposit is referred to herein as "the deposited strain" or as "the DNA of the deposited strain."

The deposited strain contains the full length ribG gene. The sequence of the polynucleotides contained in the deposited strain, as well as the amino acid sequence of the polypeptide encoded thereby, are controlling in the event of any conflict with any description of sequences herein.

The deposit of the deposited strain has been made under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for Purposes of Patent Procedure. The strain will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. The deposited strain is provided merely as convenience to those of skill in the art and is not an admission that a deposit is required for enablement, such as that required under 35 U.S.C. §112.

A license may be required to make, use or sell the deposited strain, and compounds derived therefrom, and no such license is hereby granted.

One aspect of the invention there is provided an isolated nucleic acid molecule encoding a mature polypeptide expressible by the *Staphylococcus aureus* WCUH 29 strain contained in the deposited strain. Further provided by the invention are ribG nucleotide sequences of the DNA in the deposited strain and amino acid sequences encoded thereby. Also provided by the invention are ribG polypeptide sequences isolated from the deposited strain and amino acid sequences derived therefrom.

Polypeptides

The polypeptides of the invention include a polypeptide of Table 1 [SEQ ID NO:2 or 4] (in particular the mature polypeptide) as well as polypeptides and fragments, particularly those which have the biological activity of ribG, and also those which have at least 70% identity to a polypeptide of Table 1 [SEQ ID NO:1 or 3]or the relevant portion, preferably at least 80% identity to a polypeptide of Table 1 [SEQ ID NO:2 or 4and more preferably at least 90% similarity (more preferably at least 90% identity) to a polypeptide of Table 1 [SEQ ID NO:2 or 4] and still more preferably at least 95% similarity (still more preferably at least 95% identity) to a polypeptide of Table 1 [SEQ ID NO:2 or 4] and also include portions of such polypeptides with

such portion of the polypeptide generally containing at least 30 amino acids and more preferably at least 50 amino acids.

The invention also includes polypeptides of the formula:

$$X\text{-}(R_1)_m\text{-}(R_2)\text{-}(R_3)_n\text{-}Y$$

wherein, at the amino terminus, X is hydrogen, and at the carboxyl terminus, Y is hydrogen or a metal, R_1 and R_3 are any amino acid residue, m is an integer between 1 and 1000 or zero, n is an integer between 1 and 1000 or zero, and R_2 is an amino acid sequence of the invention, particularly an amino acid sequence selected from Table 1. In the formula above R_2 is oriented so that its amino terminal residue is at the left, bound to R_1, and its carboxy terminal residue is at the right, bound to R_3. Any stretch of amino acid residues denoted by either R group, where m and/or n is greater than 1, may be either a heteropolymer or a homopolymer, preferably a heteropolymer.

A fragment is a variant polypeptide having an amino acid sequence that entirely is the same as part but not all of the amino acid sequence of the aforementioned polypeptides. As with ribG polypeptides fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region, a single larger polypeptide.

Preferred fragments include, for example, truncation polypeptides having a portion of an amino acid sequence of Table 1 [SEQ ID NO:2 or 4], or of variants thereof, such as a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus. Degradation forms of the polypeptides of the invention in a host cell, particularly a *Staphylococcus aureus*, are also preferred. Further preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions.

Also preferred are biologically active fragments which are those fragments that mediate activities of ribG, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those fragments that are antigenic or immunogenic in an animal, especially in a human. Particularly preferred are fragments

comprising receptors or domains of enzymes that confer a function essential for viability of *Staphylococcus aureus* or the ability to initiate, or maintain cause disease in an individual, particularly a human.

Variants that are fragments of the polypeptides of the invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, these variants may be employed as intermediates for producing the full-length polypeptides of the invention.

In addition to the standard single and triple letter representations for amino acids, the term "X" or "Xaa" may also be used in describing certain polypeptides of the invention. "X" and "Xaa" mean that any of the twenty naturally occuring amino acids may appear at such a designated position in the polypeptide sequence.

Polynucleotides

Another aspect of the invention relates to isolated polynucleotides, including the full length gene, that encode the ribG polypeptide having a deduced amino acid sequence of Table 1 [SEQ ID NO:2 or 4] and polynucleotides closely related thereto and variants thereof.

Using the information provided herein, such as a polynucleotide sequence set out in Table 1 [SEQ ID NO:1 or 3], a polynucleotide of the invention encoding ribG polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing chromosomal DNA fragments from bacteria using *Staphylococcus aureus* WCUH 29 cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a sequence given in Table 1 [SEQ ID NO:1 or 3], typically a library of clones of chromosomal DNA of *Staphylococcus aureus* WCUH 29 in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent conditions. By sequencing the individual clones thus identified with sequencing primers designed from the original sequence it is then possible to extend the sequence in both directions to determine the full gene sequence. Conveniently, such sequencing is performed using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening

By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Illustrative of the invention, the polynucleotide set out in Table 1 [SEQ ID NO:1 or 3] was discovered in a DNA library derived from *Staphylococcus aureus* WCUH 29.

The DNA sequence set out in Table 1 [SEQ ID NO:1 or 3] contains an open reading frame encoding a protein having about the number of amino acid residues set forth in Table 1 [SEQ ID NO:2 or 4] with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known in the art. The polynucleotide of SEQ ID NO: 1, between nucleotide number 1 and the stop codon which begins at nucleotide number 1027 of SEQ ID NO:1, encodes the polypeptide of SEQ ID NO:2.

RibG of the invention is structurally related to other proteins of the riboflavin specific deaminase family.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence in Table 1 [SEQ ID NO:1 or 3]. Also provided by the invention is the coding sequence for the mature polypeptide or a fragment thereof, by itself as well as the coding sequence for the mature polypeptide or a fragment in reading frame with other coding sequence, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence. The polynucleotide may also contain non-coding sequences, including for example, but not limited to non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences, termination signals, ribosome binding sites, sequences that stabilize mRNA, introns, polyadenylation signals, and additional coding sequence which encode additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc. Natl. Acad. Sci., USA 86:* 821-824 (1989), or an HA tag (Wilson *et al.*, *Cell 37:* 767 (1984). Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

A preferred embodiment of the invention is a polynucleotide of comprising nucleotide 1 to the nucleotide immediately upstream of or including nucleotide 1027 set forth in SEQ ID NO:1 of Table 1, both of which encode the ribG polypeptide.

The invention also includes polynucleotides of the formula:

$$X\text{-}(R_1)_m\text{-}(R_2)\text{-}(R_3)_n\text{-}Y$$

wherein, at the 5' end of the molecule, X is hydrogen, and at the 3' end of the molecule, Y is hydrogen or a metal, R_1 and R_3 is any nucleic acid residue, m is an integer between 1 and 3000 or zero , n is an integer between 1 and 3000 or zero, and R_2 is a nucleic acid sequence of the invention, particularly a nucleic acid sequence selected from Table 1. In the polynucleotide formula above R_2 is oriented so that its 5' end residue is at the left, bound to R_1, and its 3' end residue is at the right, bound to R_3. Any stretch of nucleic acid residues denoted by either R group, where m and/or n is greater than 1, may be either a heteropolymer or a homopolymer, preferably a heteropolymer. In a preferred embodiment m and/or n is an integer between 1 and 1000.

It is most preferred that the polynucleotides of the inventions are derived from *Staphylococcus aureus*, however, they may preferably be obtained from organisms of the same taxonomic genus. They may also be obtained, for example, from organisims of the same taxonomic family or order.

The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Staphylococcus aureus* ribG having an amino acid sequence set out in Table 1 [SEQ ID NO:2 or 4]. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, interrupted by integrated phage or an insertion sequence or editing) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode for variants of the polypeptide having a deduced amino acid sequence of Table 1 [SEQ ID NO:2 or 4]. Variants that are fragments of the polynucleotides of the invention may be used to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding ribG variants, that have the amino acid sequence of ribG polypeptide of Table 1 [SEQ ID NO:2 or 4] in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, that do not alter the properties and activities of ribG.

Further preferred embodiments of the invention are polynucleotides that are at least 70% identical over their entire length to a polynucleotide encoding ribG polypeptide having an amino acid sequence set out in Table 1 [SEQ ID NO:2 or 4], and polynucleotides that are complementary to such polynucleotides. Alternatively, most highly preferred are polynucleotides that comprise a region that is at least 80% identical over its entire length to a polynucleotide encoding ribG polypeptide and polynucleotides complementary thereto. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides that encode polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of Table 1 [SEQ ID NO:1 or 3].

The invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. An example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein.

The invention also provides a polynucleotide consisting essentially of a polynucleotide sequence obtainable by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:1 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:1 or

a fragment thereof; and isolating said DNA sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers described elsewhere herein.

As discussed additionally herein regarding polynucleotide assays of the invention, for instance, polynucleotides of the invention as discussed above, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding ribG and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the ribG gene. Such probes generally will comprise at least 15 bases. Preferably, such probes will have at least 30 bases and may have at least 50 bases. Particularly preferred probes will have at least 30 bases and will have 50 bases or less.

For example, the coding region of the ribG gene may be isolated by screening using a DNA sequence provided in Table 1 [SEQ ID NO: 1 or 3] to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

The polynucleotides and polypeptides of the invention may be employed, for example, as research reagents and materials for discovery of treatments of and diagnostics for disease, particularly human disease, as further discussed herein relating to polynucleotide assays.

Polynucleotides of the invention that are oligonucleotides derived from the sequences of Table 1 [SEQ ID NOS:1 or 2 or 3 or 4] may be used in the processes herein as described, but preferably for PCR, to determine whether or not the polynucleotides identified herein in whole or in part are transcribed in bacteria in infected tissue. It is recognized that such sequences will also have utility in diagnosis of the stage of infection and type of infection the pathogen has attained.

The invention also provides polynucleotides that may encode a polypeptide that is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, may allow protein transport, may lengthen or shorten protein half-life or may facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in vivo*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

A precursor protein, having the mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

In addition to the standard A, G, C, T/U representations for nucleic acid bases, the term "N" may also be used in describing certain polynucleotides of the invention. "N" means that any of the four DNA or RNA bases may appear at such a designated position in the DNA or RNA sequence, except it is preferred that N is not a base that when taken in combination with adjacent nucleotide positions, when read in the correct reading frame, would have the effect of generating a premature termination codon in such reading frame.

In sum, a polynucleotide of the invention may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor of a mature protein having one or more prosequences that are not the leader sequences of a preprotein, or a preproprotein, which is a precursor to a proprotein, having a leader sequence and one or more prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

Vectors, host cells, expression

The invention also relates to vectors that comprise a polynucleotide or polynucleotides of the invention, host cells that are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof or polynucleotides of the invention. Introduction of a polynucleotide into the host cell can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY*, (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), such as, calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction and infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, enterococci *E. coli*, streptomyces and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used to produce the polypeptides of the invention. Such vectors include, among others, chromosomal, episomal and virus-derived vectors, *e.g.*, vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression system constructs may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides and/or to express a polypeptide in a host may be used for expression in this regard. The appropriate DNA sequence may be inserted into the expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al., MOLECULAR CLONING, A LABORATORY MANUAL, (supra)*.

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

Polypeptides of the invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

This invention is also related to the use of the ribG polynucleotides of the invention for use as diagnostic reagents. Detection of ribG in a eukaryote, particularly a mammal, and especially a human, will provide a diagnostic method for diagnosis of a disease. Eukaryotes (herein also "individual(s)"), particularly mammals, and especially humans, particularly those infected or suspected to be infected with an organism comprising the ribG gene may be detected at the nucleic acid level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from an infected individual's cells and tissues, such as bone, blood, muscle, cartilage, and skin. Genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification technique prior to analysis. RNA, cDNA and genomic DNA may also be used in the same ways. Using amplification, characterization of the species and strain of prokaryote present in an individual, may be made by an analysis of the genotype of the prokaryote gene. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the genotype of a reference sequence. Point mutations can be identified by hybridizing amplified DNA to labeled ribG polynucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in the electrophoretic mobility of the DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, *e.g.*, Myers et al., *Science, 230:* 1242 (1985). Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase and S1 protection or a chemical cleavage method. See, *e.g.*, Cotton et al., *Proc. Natl. Acad. Sci., USA, 85:* 4397-4401 (1985).

Cells carrying mutations or polymorphisms in the gene of the invention may also be detected at the DNA level by a variety of techniques, to allow for serotyping, for example. For example, RT-PCR can be used to detect mutations. It is particularly preferred to used RT-PCR in conjunction with automated detection systems, such as, for example, GeneScan. RNA, cDNA or genomic DNA may also be used for the same purpose, PCR or RT-PCR. As an example, PCR primers complementary to a nucleic acid encoding ribG can be used to identify and analyze mutations. Examples of representative primers are shown below in Table 2.

Table 2

Primers for amplification of ribG polynucleotides

SEQ ID NO	PRIMER SEQUENCE
5	5'-ATGAAGGTAGGATTGTTGGTA-3'
6	5'-AGTCTTGGTATAATTGTGATGC-3'

The invention also includes primers of the formula:

$$X\text{-}(R_1)_m\text{-}(R_2)\text{-}(R_3)_n\text{-}Y$$

wherein, at the 5' end of the molecule, X is hydrogen, and at the 3' end of the molecule, Y is hydrogen or a metal, R_1 and R_3 is any nucleic acid residue, m is an integer between 1 and 20 or zero , n is an integer between 1 and 20 or zero, and R_2 is a primer sequence of the invention, particularly a primer sequence selected from Table 2. In the polynucleotide formula above R_2 is oriented so that its 5' end residue is at the left, bound to R_1, and its 3' end residue is at the right, bound to R_3. Any stretch of nucleic acid residues denoted by either R group, where m and/or n is greater than 1, may be either a heteropolymer or a homopolymer, preferably a heteropolymer being complementary to a region of a polynucleotide of Table 1. In a preferred embodiment m and/or n is an integer between 1 and 10.

The invention further provides these primers with 1, 2, 3 or 4 nucleotides removed from the 5' and/or the 3' end. These primers may be used for, among other things, amplifying ribG DNA isolated from a sample derived from an individual. The primers may be used to amplify the gene isolated from an infected individual such that the gene may then be subject to various techniques for elucidation of the DNA sequence. In this way, mutations in the DNA sequence may be detected and used to diagnose infection and to serotype and/or classify the infectious agent.

The invention further provides a process for diagnosing, disease, preferably bacterial infections, more preferably infections by *Staphylococcus aureus*, comprising determining from a sample derived from an individual a increased level of expression of polynucleotide having a sequence of Table 1 [SEQ ID NO: 1 or 3]. Increased or decreased expression of ribG polynucleotide can be measured using any on of the methods well known in the art for the quantation of polynucleotides, such as, for example, amplification, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods.

In addition, a diagnostic assay in accordance with the invention for detecting over-expression of ribG protein compared to normal control tissue samples may be used to detect the presence of an infection, for example. Assay techniques that can be used to determine levels of a ribG protein, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Antibodies

The polypeptides of the invention or variants thereof, or cells expressing them can be used as an immunogen to produce antibodies immunospecific for such polypeptides. "Antibodies" as used herein includes monoclonal and polyclonal antibodies, chimeric, single chain, simianized antibodies and humanized antibodies, as well as Fab fragments, including the products of an Fab immunolglobulin expression library.

Antibodies generated against the polypeptides of the invention can be obtained by administering the polypeptides or epitope-bearing fragments, analogues or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique known in the art that provides antibodies produced by continuous cell line cultures can be used. Examples include various techniques, such as those in Kohler, G. and Milstein, C., *Nature 256:* 495-497 (1975); Kozbor *et al., Immunology Today 4:* 72 (1983); Cole et al., pg. 77-96 in *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, Alan R. Liss, Inc. (1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies.

Alternatively phage display technology may be utilized to select antibody genes with binding activities towards the polypeptide either from repertoires of PCR amplified v-genes of lymphocytes from humans screened for possessing anti-ribG or from naive libraries (McCafferty, J. et al., (1990), Nature 348, 552-554; Marks, J. et al., (1992) Biotechnology *10*, 779-783). The affinity of these antibodies can also be improved by chain shuffling (Clackson, T. et al., (1991) Nature 352, 624-628).

If two antigen binding domains are present each domain may be directed against a different epitope - termed 'bispecific' antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptides to purify the polypeptides by affinity chromatography.

Thus, among others, antibodies against ribG- polypeptide may be employed to treat infections, particularly bacterial infections.

Polypeptide variants include antigenically, epitopically or immunologically equivalent variants that form a particular aspect of this invention. The term "antigenically equivalent derivative" as used herein encompasses a polypeptide or its equivalent which will be specifically recognized by certain antibodies which, when raised to the protein or polypeptide according to the invention, interfere with the immediate physical interaction between pathogen and mammalian host. The term "immunologically equivalent derivative" as used herein encompasses a peptide or its equivalent which when used in a suitable formulation to raise antibodies in a vertebrate, the antibodies act to interfere with the immediate physical interaction between pathogen and mammalian host.

The polypeptide, such as an antigenically or immunologically equivalent derivative or a fusion protein thereof is used as an antigen to immunize a mouse or other animal such as a rat or chicken. The fusion protein may provide stability to the polypeptide. The antigen may be associated, for example by conjugation, with an immunogenic carrier protein for example bovine serum albumin (BSA) or keyhole limpet haemocyanin (KLH). Alternatively a multiple antigenic peptide comprising multiple copies of the protein or polypeptide, or an antigenically or immunologically equivalent polypeptide thereof may be sufficiently antigenic to improve immunogenicity so as to obviate the use of a carrier.

Preferably, the antibody or variant thereof is modified to make it less immunogenic in the individual. For example, if the individual is human the antibody may most preferably be "humanized"; where the complimentarity determining region(s) of the hybridoma-derived antibody has been transplanted into a human monoclonal antibody , for example as described in Jones, P. et al. (1986), *Nature* 321, 522-525 or Tempest et al., (1991) *Biotechnology* 9, 266-273.

The use of a polynucleotide of the invention in genetic immunization will preferably employ a suitable delivery method such as direct injection of plasmid DNA into muscles (Wolff et al., Hum Mol Genet 1992, 1:363, Manthorpe et al., Hum. Gene Ther. 1963:4, 419), delivery of DNA complexed with specific protein carriers (Wu et al., *J Biol Chem.* 1989:

264,16985), coprecipitation of DNA with calcium phosphate (Benvenisty & Reshef, *PNAS USA*, 1986:83,9551), encapsulation of DNA in various forms of liposomes (Kaneda et al., *Science* 1989:243,375), particle bombardment (Tang et al., *Nature* 1992, 356:152, Eisenbraun et al., *DNA Cell Biol* 1993, 12:791) and *in vivo* infection using cloned retroviral vectors (Seeger et al., *PNAS USA* 1984:81,5849).

Antagonists and agonists - assays and molecules

Polypeptides of the invention may also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See, *e.g.*, Coligan *et al.*, *Current Protocols in Immunology 1(2):* Chapter 5 (1991).

The invention also provides a method of screening compounds to identify those which enhance (agonist) or block (antagonist) the action of ribG polypeptides or polynucleotides, particularly those compounds that are bacteriostatic and/or bacteriocidal. The method of screening may involve high-throughput techniques. For example, to screen for agonists or antagoists, a synthetic reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, comprising ribG polypeptide and a labeled substrate or ligand of such polypeptide is incubated in the absence or the presence of a candidate molecule that may be a ribG agonist or antagonist. The ability of the candidate molecule to agonize or antagonize the ribG polypeptide is reflected in decreased binding of the labeled ligand or decreased production of product from such substrate. Molecules that bind gratuitously, *i.e.*, without inducing the effects of ribG polypeptide are most likely to be good antagonists. Molecules that bind well and increase the rate of product production from substrate are agonists. Detection of the rate or level of production of product from substrate may be enhanced by using a reporter system. Reporter systems that may be useful in this regard include but are not limited to colorimetric labeled substrate converted into product, a reporter gene that is responsive to changes in ribG polynucleotide or polypeptide activity, and binding assays known in the art.

Another example of an assay for ribG antagonists is a competitive assay that combines ribG and a potential antagonist with ribG-binding molecules, recombinant ribG binding molecules, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. RibG can be labeled, such as by radioactivity or a

colorimetric compound, such that the number of ribG molecules bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to a polynucleotide or polypeptide of the invention and thereby inhibit or extinguish its activity. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a binding molecule, without inducing ribG-induced activities, thereby preventing the action of ribG by excluding ribG from binding.

Potential antagonists include a small molecule that binds to and occupies the binding site of the polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules. Other potential antagonists include antisense molecules (see Okano, *J. Neurochem. 56:* 560 (1991); *OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION*, CRC Press, Boca Raton, FL (1988), for a description of these molecules). Preferred potential antagonists include compounds related to and variants of ribG.

Each of the DNA sequences provided herein may be used in the discovery and development of antibacterial compounds. The encoded protein, upon expression, can be used as a target for the screening of antibacterial drugs. Additionally, the DNA sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other translation facilitating sequences of the respective mRNA can be used to construct antisense sequences to control the expression of the coding sequence of interest.

The invention also provides the use of the polypeptide, polynucleotide or inhibitor of the invention to interfere with the initial physical interaction between a pathogen and mammalian host responsible for sequelae of infection. In particular the molecules of the invention may be used: in the prevention of adhesion of bacteria, in particular gram positive bacteria, to mammalian extracellular matrix proteins on in-dwelling devices or to extracellular matrix proteins in wounds; to block ribG protein-mediated mammalian cell invasion by, for example, initiating phosphorylation of mammalian tyrosine kinases (Rosenshine *et al.*, *Infect. Immun. 60*:2211 (1992); to block bacterial adhesion between mammalian extracellular matrix

proteins and bacterial ribG proteins that mediate tissue damage and; to block the normal progression of pathogenesis in infections initiated other than by the implantation of in-dwelling devices or by other surgical techniques.

The antagonists and agonists of the invention may be employed, for instance, to inhibit and treat diseases.

Helicobacter pylori (herein *H. pylori*) bacteria infect the stomachs of over one-third of the world's population causing stomach cancer, ulcers, and gastritis (International Agency for Research on Cancer (1994) Schistosomes, Liver Flukes and Helicobacter Pylori (International Agency for Research on Cancer, Lyon, France; http://www.uicc.ch/ecp/ecp2904.htm). Moreover, the international Agency for Research on Cancer recently recognized a cause-and-effect relationship between *H. pylori* and gastric adenocarcinoma, classifying the bacterium as a Group I (definite) carcinogen. Preferred antimicrobial compounds of the invention (agonists and antagonists of ribG) found using screens provided by the invention, particularly broad-spectrum antibiotics, should be useful in the treatment of *H. pylori* infection. Such treatment should decrease the advent of *H. pylori*-induced cancers, such as gastrointestinal carcinoma. Such treatment should also cure gastric ulcers and gastritis.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in an individual, particularly a mammal which comprises inoculating the individual with ribG, or a fragment or variant thereof, adequate to produce antibody and/ or T cell immune response to protect said individual from infection, particularly bacterial infection and most particularly *Staphylococcus aureus* infection. Also provided are methods whereby such immunological response slows bacterial replication. Yet another aspect of the invention relates to a method of inducing immunological response in an individual which comprises delivering to such individual a nucleic acid vector to direct expression of ribG, or a fragment or a variant thereof, for expressing ribG, or a fragment or a variant thereof *in vivo* in order to induce an immunological response, such as, to produce antibody and/ or T cell immune response, including, for example, cytokine-producing T cells or cytotoxic T cells, to protect said individual from disease, whether that disease is already established within the individual or not. One way of administering the gene is by accelerating it into the desired cells as a coating on

particles or otherwise. Such nucleic acid vector may comprise DNA, RNA, a modified nucleic acid, or a DNA/RNA hybrid.

A further aspect of the invention relates to an immunological composition which, when introduced into an individual capable or having induced within it an immunological response, induces an immunological response in such individual to a ribG or protein coded therefrom, wherein the composition comprises a recombinant ribG or protein coded therefrom comprising DNA which codes for and expresses an antigen of said ribG or protein coded therefrom. The immunological response may be used therapeutically or prophylactically and may take the form of antibody immunity or cellular immunity such as that arising from CTL or CD4+ T cells.

A ribG polypeptide or a fragment thereof may be fused with co-protein which may not by itself produce antibodies, but is capable of stabilizing the first protein and producing a fused protein which will have immunogenic and protective properties. Thus fused recombinant protein, preferably further comprises an antigenic co-protein, such as lipoprotein D from *Hemophilus influenzae*, Glutathione-S-transferase (GST) or beta-galactosidase, relatively large co-proteins which solubilize the protein and facilitate production and purification thereof. Moreover, the co-protein may act as an adjuvant in the sense of providing a generalized stimulation of the immune system. The co-protein may be attached to either the amino or carboxy terminus of the first protein.

Provided by this invention are compositions, particularly vaccine compositions, and methods comprising the polypeptides or polynucleotides of the invention and immunostimulatory DNA sequences, such as those described in Sato, Y. *et al.* Science 273: 352 (1996).

Also, provided by this invention are methods using the described polynucleotide or particular fragments thereof which have been shown to encode non-variable regions of bacterial cell surface proteins in DNA constructs used in such genetic immunization experiments in animal models of infection with *Staphylococcus aureus* will be particularly useful for identifying protein epitopes able to provoke a prophylactic or therapeutic immune response. It is believed that this approach will allow for the subsequent preparation of monoclonal antibodies of particular value from the requisite organ of the animal successfully resisting or clearing infection for the development of prophylactic agents or therapeutic

treatments of bacterial infection, particularly *Staphylococcus aureus* infection, in mammals, particularly humans.

The polypeptide may be used as an antigen for vaccination of a host to produce specific antibodies which protect against invasion of bacteria, for example by blocking adherence of bacteria to damaged tissue. Examples of tissue damage include wounds in skin or connective tissue caused, e.g., by mechanical, chemical or thermal damage or by implantation of indwelling devices, or wounds in the mucous membranes, such as the mouth, mammary glands, urethra or vagina.

The invention also includes a vaccine formulation which comprises an immunogenic recombinant protein of the invention together with a suitable carrier. Since the protein may be broken down in the stomach, it is preferably administered parenterally, including, for example, administration that is subcutaneous, intramuscular, intravenous, or intradermal. Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation insotonic with the bodily fluid, preferably the blood, of the individual; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

While the invention has been described with reference to certain ribG protein, it is to be understood that this covers fragments of the naturally occurring protein and similar proteins with additions, deletions or substitutions which do not substantially affect the immunogenic properties of the recombinant protein.

Compositions, kits and administration

The invention also relates to compositions comprising the polynucleotide or the polypeptides discussed above or their agonists or antagonists. The polypeptides of the invention may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to a subject.

Such compositions comprise, for instance, a media additive or a therapeutically effective amount of a polypeptide of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration. The invention further relates to diagnostic and pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

Alternatively the composition may be formulated for topical application for example in the form of ointments, creams, lotions, eye ointments, eye drops, ear drops, mouthwash, impregnated dressings and sutures and aerosols, and may contain appropriate conventional additives, including, for example, preservatives, solvents to assist drug penetration, and emollients in ointments and creams. Such topical formulations may also contain compatible conventional carriers, for example cream or ointment bases, and ethanol or oleyl alcohol for lotions. Such carriers may constitute from about 1% to about 98% by weight of the formulation; more usually they will constitute up to about 80% by weight of the formulation.

For administration to mammals, and particularly humans, it is expected that the daily dosage level of the active agent will be from 0.01 mg/kg to 10 mg/kg, typically around 1 mg/kg. The physician in any event will determine the actual dosage which will be most suitable for an individual and will vary with the age, weight and response of the particular individual. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

In-dwelling devices include surgical implants, prosthetic devices and catheters, i.e., devices that are introduced to the body of an individual and remain in position for an extended time. Such devices include, for example, artificial joints, heart valves, pacemakers, vascular grafts, vascular catheters, cerebrospinal fluid shunts, urinary catheters, continuous ambulatory peritoneal dialysis (CAPD) catheters.

The composition of the invention may be administered by injection to achieve a systemic effect against relevant bacteria shortly before insertion of an in-dwelling device. Treatment may be continued after surgery during the in-body time of the device. In addition, the composition could also be used to broaden perioperative cover for any surgical technique to prevent bacterial wound infections, especially *Staphylococcus aureus* wound infections.

Many orthopaedic surgeons consider that humans with prosthetic joints should be considered for antibiotic prophylaxis before dental treatment that could produce a bacteremia. Late deep infection is a serious complication sometimes leading to loss of the prosthetic joint and is accompanied by significant morbidity and mortality. It may therefore be possible to extend the use of the active agent as a replacement for prophylactic antibiotics in this situation.

In addition to the therapy described above, the compositions of this invention may be used generally as a wound treatment agent to prevent adhesion of bacteria to matrix proteins exposed in wound tissue and for prophylactic use in dental treatment as an alternative to, or in conjunction with, antibiotic prophylaxis.

Alternatively, the composition of the invention may be used to bathe an indwelling device immediately before insertion. The active agent will preferably be present at a concentration of 1μg/ml to 10mg/ml for bathing of wounds or indwelling devices.

A vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response. A suitable unit dose for vaccination is 0.5-5 microgram/kg of antigen, and such dose is preferably administered 1-3 times and with an interval of 1-3 weeks. With the indicated dose range, no adverse toxicological effects will be observed with the compounds of the invention which would preclude their administration to suitable individuals.

Each reference disclosed herein is incorporated by reference herein in its entirety. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety.

GLOSSARY

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"Disease(s)" means and disease caused by or related to infection by a bacteria, including disease, such as, infections of the upper respiratory tract (e.g., otitis media, bacterial tracheitis, acute epiglottitis, thyroiditis), lower respiratory (e.g., empyema, lung abscess), cardiac (e.g., infective endocarditis), gastrointestinal (e.g., secretory diarrhoea, splenic absces, retroperitoneal abscess), CNS (e.g., cerebral abscess), eye (e.g., blepharitis, conjunctivitis, keratitis, endophthalmitis, preseptal and orbital cellulitis, darcryocystitis), kidney and urinary tract (e.g., epididymitis, intrarenal and perinephric absces, toxic shock syndrome), skin (e.g., impetigo, folliculitis, cutaneous abscesses, cellulitis, wound infection, bacterial myositis) bone and joint (e.g., septic arthritis, osteomyelitis).

"Host cell" is a cell which has been transformed or transfected, or is capable of transformation or transfection by an exogenous polynucleotide sequence.

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM *J. Applied Math., 48:* 1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., *Nucleic Acids Research 12(1):* 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. et

al., *J. Molec. Biol. 215:* 403-410 (1990). The BLAST X program is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., *et al.*, NCBI NLM NIH Bethesda, MD 20894; Altschul, S., *et al.*, *J. Mol. Biol. 215*: 403-410 (1990). As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO: 1 it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO: 1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5′ or 3′ terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. Analogously , by a polypeptide having an amino acid sequence having at least, for example, 95% identity to a reference amino acid sequence of SEQ ID NO:2 is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO: 2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

"Isolated" means altered "by the hand of man" from its natural state, *i.e.*, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is

"isolated", as the term is employed herein. Moreover, a polynucleotide or polypeptide that is introduced into an organism by transformation, genetic manipulation or by any other recombinant method is "isolated" even if it is still present in said organism, which organism may be living or non-living.

"Polynucleotide(s)" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotide(s)" include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions or single-, double- and triple-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded, or triple-stranded regions, or a mixture of single- and double-stranded regions. In addition, "polynucleotide" as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. As used herein, the term "polynucleotide(s)" also includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotide(s)" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term "polynucleotide(s)" as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including, for example, simple and complex cells. "Polynucleotide(s)" also embraces short polynucleotides often referred to as oligonucleotide(s).

"Polypeptide(s)" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds. "Polypeptide(s)" refers to both short chains, commonly referred to as peptides, oligopeptides and oligomers and to longer chains generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene encoded amino acids. "Polypeptide(s)" include those modified either by natural processes, such

as processing and other post-translational modifications, but also by chemical modification techniques. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature, and they are well known to those of skill in the art. It will be appreciated that the same type of modification may be present in the same or varying degree at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains, and the amino or carboxyl termini. Modifications include, for example, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins, such as arginylation, and ubiquitination. See, for instance, *PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES*, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993) and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in *POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS*, B. C. Johnson, Ed., Academic Press, New York (1983); Seifter et al., *Meth. Enzymol. 182*:626-646 (1990) and Rattan et al., *Protein Synthesis: Posttranslational Modifications and Aging*, Ann. N.Y. Acad. Sci. 663: 48-62 (1992). Polypeptides may be branched or cyclic, with or without branching. Cyclic, branched and branched circular polypeptides may result from post-translational natural processes and may be made by entirely synthetic methods, as well.

"Variant(s)" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide

changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques, by direct synthesis, and by other recombinant methods known to skilled artisans.

EXAMPLES

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples are illustrative, but do not limit the invention.

Example 1 Strain selection, Library Production and Sequencing

The polynucleotide having a DNA sequence given in Table 1 [SEQ ID NO:1 or 3] was obtained from a library of clones of chromosomal DNA of *Staphylococcus aureus* in *E. coli.* The sequencing data from two or more clones containing overlapping *Staphylococcus aureus* DNAs was used to construct the contiguous DNA sequence in SEQ ID NO:1. Libraries may be prepared by routine methods, for example:

Methods 1 and 2 below.

Total cellular DNA is isolated from *Staphylococcus aureus* WCUH 29 according to standard procedures and size-fractionated by either of two methods.

Method 1

Total cellular DNA is mechanically sheared by passage through a needle in order to size-fractionate according to standard procedures. DNA fragments of up to 11kbp in size are rendered blunt by treatment with exonuclease and DNA polymerase, and EcoRI linkers added. Fragments are ligated into the vector Lambda ZapII that has been cut with EcoRI, the library packaged by standard procedures and *E.coli* infected with the packaged library. The library is amplified by standard procedures.

Method 2

Total cellular DNA is partially hydrolyzed with a one or a combination of restriction enzymes appropriate to generate a series of fragments for cloning into library vectors (e.g., RsaI, PalI, AluI, Bshl235I), and such fragments are size-fractionated according to standard procedures. EcoRI linkers are ligated to the DNA and the fragments then ligated into the vector Lambda ZapII that have been cut with EcoRI, the library packaged by standard procedures, and *E.coli* infected with the packaged library. The library is amplified by standard procedures.

Example 2 ribG Characterization

Riboflavin Biosynthesis Operon

This ORF is part of an operon which encodes genes *ribG*, *ribB*, *ribA* and *ribH*. Gene *ribG* starts at nucleotide 1 and ends at nucleotide 1029. Gene *ribB* starts at nucleotide 1036 and ends at nucleotide 1668. Gene *ribA* starts at nucleotide 1679 and ends at nucleotide 2860. Gene *ribH* starts at nucleotide 2873 and ends at nucleotide 3337. The operon sequence below is SEQ ID NO:7.

ATGGATTATGCGATTCAACTTGCAAATATGGTACAAGGTCAAACAGGTGTTAATCCACCCGTTGGCGCTG
TTGTAGTTAATGAAGGTAGGATTGTTGGTATTGGTGCACACTTGAGAAAAGGTGACAAGCATGCGGAGGT
TCAAGCACTTGATATGGCACAACAAAATGCTGAAGGTGCGACGATTTATATTACGTTAGAGCCATGTAGT
CATTTTGGTTCAACACCACCCTGTGTTAACAAAATTATTGATTGTAAGATAGCAAAAGTAGTATACGCAA
CAAAAGACAATTCGTTAGACACACATGGTGATGAGACGTTACGGGCTCACGGTATTGAGGTTGAATGCGT
TGATGATGAACGGGCATCACAATTATACCAAGACTTTTTAAAGCAAAAGCAAAGCAACTGCCACAAATT
ACAGTGAAAGTATCTGCAAGTTTAGATGGTAAACAAGCGAATGATAATGGACAAAGTCAATGGATTACTA
ACAAAGAGGTTAAACAAGATGTCTATAAGTTAAGACATCGACACGACGCAGTGTTAACTGGAAGACGTAC
AGTTGAATTAGATGATCCACAATATACTACACGTATTCAAGATGGAAAAAACCCTATAAAAGTAATATTG
TCTAAGTCTGGGAATATTCATTTTAATCAGCAAATTTATCAAGATGAATCAACACCAATTTGGATATATA
CTGAAAATCCAAATTTAACAAGCAATCAAACACATATTGAAATTATTTACTTGAAGTCTTGTGATTTAAC
AACAATTCTTCACAATTTATATAAAAGAGGAGTTGGAACTTTGCTAGTCGAGGCAGGTCCAACCACTACT
TCAGAATTCTCCATCTATTATATAGATGAATTTATTCTCTATTATGCCCCGAAATTAATTGGCGGATCTG
GAAATTATCAATTTTATCAAACAAATGATGTGATTGAGATACCAGATGCGAACCAATTTGAAATTGTTCA
TTCCGAGTTATTAAATCAAAATGTTAAATTAACTTTACGAAAGAAGTGATGATGCATGTTTACTGGCATC
GTTGAAGAAATAGGTGTCGTTAAAAGTGTTCAAATTCGTCAATCAGTCAGGACGATTGAAATCGAAGCAC

ATAAGATTACGGCAGATATGCATATTGGTGATTCCATCAGTGTTAATGGTGCATGTTTAACAGTGATTGA
TTTTGATCAGACATCTTTTACTGTTCAAGTAATTAAAAGCACTGAAAATAAAACCTATTTAGCAGATGTT
AAGCGACAATCAGAAGTAAATTTAGAGCGTGCCATGAGTGGTAACGGTAGGTTTGGTGGACATTTTGTCC
TCGGTCATGTAGATGAACTAGGAACAGTTT
CAAAAATAAATGAAACAGCCAATGCCAAAATTATTACGATTCAATGTAGCCAACATATTAATAATCAGTT
AGTTAAGCAAGGGTCTATTACTGTGGATGGTGTAAGTCTAACGGTATTTGATAAGCATGATAACAGTTTT
GACATTCATCTTATTCCAGAAACGAGGCGTTCAACGATTTTATCATCCAAAAAATTAGGAGATAAAGTAC
ATTTAGAAACAGACGTTTTGTTTAAATATGTTGAAAATATTTTAAATAAAGATAAAGACCAATTATCTGT
AGATAAATTAAGAGCATTTGGTTTTTAGGAGGGGTAGCATGCAATTCGATAATATTGACAGTGCTTTAAT
GGCTTTAAAAAATGGAGAAACAATTATTGTAGTAGATGATGAGAATCGTGAAAATGAAGGTGATTTAGTA
GCGGTTACTGAATGGATGAACGATAATACCATTAATTTTATGGCGAAAGAAGCAAGGGGATTAATATGCG
CACCAGTGTCTAAAGATATTGCACAACGTTTGGATTTGGTACAAATGGTTGATGATAACTCCGACATCTT
TGGTACGCAATTTACAGTGAGTATTGATCATGTAGATACAACAACAGGAATTAGTGCTTATGAACGTACA
TTGACTGCCAAAAAGCTCATTGATCCTAGTAGTGAAGCTAAAGATTTTAATCGTCCTGGTCATTTATTTC
CATTAGTAGCACAAGATAAAGGCGTATTAGCTAGAAATGGACACACAGAAGCGGCTGTTGATTTAGCTAA
ACTTACTGGTGCCAAGCCCGCTGGTGTCATTTGTGAGATTATGAATGATGACGGCACGATGGCGAAAGGA
CAAGATTTACAAAATTTTAAAGAAAAACATCAATTAAAGATGATTACGATTGATGATTTAATTGAATATC
GTAAAAAATTAGAACCAGAAATTGAATTTAAGGCAAAAGTGAAAATGCCTACAGATTTCGGAACATTTGA
TATGTATGGTTTTAAAGCGACATACACAGATGAAGAGATAGTTGTACTGACAAAAGGTGCAATTCGACAA
CATGAAAATGTACGCTTACATTCTGCGTGCCTTACAGGCGATATTTTCCATAGTCAACGTTGTGATTGTG
GTGCTCAACTTGAATCGTCTATGAAGTATATCAATGAACATGGTGGCATGATTATTTATCTACCTCAAGA
AGGTCGTGGCATAGGATTGTTAAACAAATTACGCGCATATGAATTAATTGAGCAAGGATATGATACAGTA
ACTGCAAATTTAGCATTAGGTTTTGATGAAGATTTACGAGATTATCATATTGCTGCACAGATTTTAAAAT
ATTTTAACATCGAACATATCAATTTATTAAGTAATAATCCAAGTAAATTTGAGGGATTAAAACAATATGG
CATTGATATTGCAGAAAGAATTGAAGTTATCGTACCAGAAACGGTACATAATCATGATTATATGGTAACG
AAAAAAATAAAAATGGGTCATTTAATATAGGAGGACTTTAACATGAATTTTGAAGGTAAATTAATTGGAA
AAGATTTGAAAGTTGCAATCGTAGTTAGTCGATTTAATGATTTTATCACTGGAAGATTACTTGAAGGTGC
AAAAGATACTTTGATTCGACATGATGTTAATGAAGACAATATTGATGTAGCATTTGTTCCTGGTGCGTTT
GAAATTCCTTTAGTAGCTAAAAAATTAGCCTCATCAGGAAATTATGATGCAATAATTACATTAGGATGCG
TAATTCGCGGTGCTACGTCTCATTATGATTATGTTTGTAATGAAGTGCGAAAGGTGTTTCTAAAGTAAAT
GATCAAACTAATGTACCAGTCATATTTGGTATTTTAACGACTGAAAGTATTGAACAAGCTGTGGAAAGAG
CAGGTACGAAAGCTGGTAATAAAGGTGCCGAAGCAGCAGTAAGTGCAATTGAAATGGCTAATTTATTAAA
ATCTATAAAAGCATAG

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Palmer, Leslie M.

(ii) TITLE OF THE INVENTION: Novel ribG

(iii) NUMBER OF SEQUENCES: 7

(iv) CORRESPONDENCE ADDRESS:
  (A) ADDRESSEE: Dechert Price & Rhoads
  (B) STREET: 4000 Bell Atlantic Tower, 1717 Arch Stre
  (C) CITY: Philadelphia
  (D) STATE: PA
  (E) COUNTRY: US
  (F) ZIP: 19103

(v) COMPUTER READABLE FORM:
  (A) MEDIUM TYPE: Diskette
  (B) COMPUTER: IBM Compatible
  (C) OPERATING SYSTEM: DOS
  (D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
  (A) APPLICATION NUMBER:
  (B) FILING DATE:
  (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
  (A) APPLICATION NUMBER:
  (B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
  (A) NAME: Dickinson, Todd Q

(B) REGISTRATION NUMBER: 28,354

(C) REFERENCE/DOCKET NUMBER: P50444-9

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 215-994-2252

(B) TELEFAX: 215-994-2222

(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1029 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
ATGGATTATG CGATTCAACT TGCAAATATG GTACAAGGTC AAACAGGTGT TAATCCACCC     60
GTTGGCGCTG TTGTAGTTAA TGAAGGTAGG ATTGTTGGTA TTGGTGCACA CTTGAGAAAA    120
GGTGACAAGC ATGCGGAGGT TCAAGCACTT GATATGGCAC AACAAAATGC TGAAGGTGCG    180
ACGATTTATA TTACGTTAGA GCCATGTAGT CATTTTGGTT CAACACCACC CTGTGTTAAC    240
AAAATTATTG ATTGTAAGAT AGCAAAAGTA GTATACGCAA CAAAAGACAA TTCGTTAGAC    300
ACACATGGTG ATGAGACGTT ACGGGCTCAC GGTATTGAGG TTGAATGCGT TGATGATGAA    360
CGGGCATCAC AATTATACCA AGACTTTTTT AAAGCAAAAG CAAAGCAACT GCCACAAATT    420
ACAGTGAAAG TATCTGCAAG TTTAGATGGT AAACAAGCGA ATGATAATGG ACAAAGTCAA    480
TGGATTACTA ACAAAGAGGT TAAACAAGAT GTCTATAAGT TAAGACATCG ACACGACGCA    540
GTGTTAACTG GAAGACGTAC AGTTGAATTA GATGATCCAC AATATACTAC ACGTATTCAA    600
GATGGAAAAA ACCCTATAAA AGTAATATTG TCTAAGTCTG GGAATATTCA TTTTAATCAG    660
CAAATTTATC AAGATGAATC AACACCAATT TGGATATATA CTGAAAATCC AAATTTAACA    720
AGCAATCAAA CACATATTGA AATTATTTAC TTGAAGTCTT GTGATTTAAC AACAATTCTT    780
CACAATTTAT ATAAAAGAGG AGTTGGAACT TTGCTAGTCG AGGCAGGTCC AACCACTACT    840
TCAGAATTCT CCATCTATTA TATAGATGAA TTTATTCTCT ATTATGCCCC GAAATTAATT    900
GGCGGATCTG GAAATTATCA ATTTTATCAA ACAAATGATG TGATTGAGAT ACCAGATGCG    960
AACCAATTTG AAATTGTTCA TTCCGAGTTA TTAAATCAAA ATGTTAAATT AACTTTACGA   1020
AAGAAGTGA                                                          1029
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 342 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Asp Tyr Ala Ile Gln Leu Ala Asn Met Val Gln Gly Gln Thr Gly
 1               5                  10                  15
Val Asn Pro Pro Val Gly Ala Val Val Val Asn Glu Gly Arg Ile Val
            20                  25                  30
Gly Ile Gly Ala His Leu Arg Lys Gly Asp Lys His Ala Glu Val Gln
        35                  40                  45
Ala Leu Asp Met Ala Gln Gln Asn Ala Glu Gly Ala Thr Ile Tyr Ile
    50                  55                  60
Thr Leu Glu Pro Cys Ser His Phe Gly Ser Thr Pro Pro Cys Val Asn
65                  70                  75                  80
Lys Ile Ile Asp Cys Lys Ile Ala Lys Val Val Tyr Ala Thr Lys Asp
                85                  90                  95
Asn Ser Leu Asp Thr His Gly Asp Glu Thr Leu Arg Ala His Gly Ile
            100                 105                 110
Glu Val Glu Cys Val Asp Asp Glu Arg Ala Ser Gln Leu Tyr Gln Asp
        115                 120                 125
Phe Phe Lys Ala Lys Ala Lys Gln Leu Pro Gln Ile Thr Val Lys Val
    130                 135                 140
Ser Ala Ser Leu Asp Gly Lys Gln Ala Asn Asp Asn Gly Gln Ser Gln
145                 150                 155                 160
Trp Ile Thr Asn Lys Glu Val Lys Gln Asp Val Tyr Lys Leu Arg His
                165                 170                 175
Arg His Asp Ala Val Leu Thr Gly Arg Arg Thr Val Glu Leu Asp Asp
            180                 185                 190
Pro Gln Tyr Thr Thr Arg Ile Gln Asp Gly Lys Asn Pro Ile Lys Val
        195                 200                 205
Ile Leu Ser Lys Ser Gly Asn Ile His Phe Asn Gln Gln Ile Tyr Gln
    210                 215                 220
Asp Glu Ser Thr Pro Ile Trp Ile Tyr Thr Glu Asn Pro Asn Leu Thr
225                 230                 235                 240
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Ser Asn Gln Thr His Ile Glu Ile Ile Tyr Leu Lys Ser Cys Asp Leu
245 250 255

Thr Thr Ile Leu His Asn Leu Tyr Lys Arg Gly Val Gly Thr Leu Leu
260 265 270

Val Glu Ala Gly Pro Thr Thr Thr Ser Glu Phe Ser Ile Tyr Tyr Ile
275 280 285

Asp Glu Phe Ile Leu Tyr Tyr Ala Pro Lys Leu Ile Gly Gly Ser Gly
290 295 300

Asn Tyr Gln Phe Tyr Gln Thr Asn Asp Val Ile Glu Ile Pro Asp Ala
305 310 315 320

Asn Gln Phe Glu Ile Val His Ser Glu Leu Leu Asn Gln Asn Val Lys
325 330 335

Leu Thr Leu Arg Lys Lys
340

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1269 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AANCACCAAT CCNATTGGGA GGNAATCCAA ATCAATNCCC GGANNCCCAA TCCAAGTTAA 60
TTAAGTCCAA GGTTTTGGAA CATTACCAAA TATGATTCCG ATGAGGTCAA ATGNCAANCG 120
GTGTTAATAA ACTACGAAAT GNTGTGNAAA TGATAGTAGA NCAAGTTGCG CATACAGTNT 180
CTCNATTATA TGATGCTTTA GAATCGAATG AGCAACAACA GCGCAGTTAC AATCAATAAT 240
TTGTAACTAG AAGATAATAA AGAGAACGCT CTATAGAGAC GAATTGAAGG TTTGATTTTA 300
ATGTCTGTTA GTAAGAATCA TATCAATGAG ATGCCTATAG TACTCAGATT ATATTAAATT 360
AAAACCGTCA TTAATTGTTT TTTTAGAAAA CATATAGTAT CATTTTAAAT GTAGTTGACA 420
TACTACGTAC TCAAATAATC TATAACAATT TCATATATAA TTCTTTCGGG GCAGGGTGAA 480
ATTCCCAACC GGCAGTAAAT AAAGCCTGCG ACCTGCTAAT ATGTTTCATA TTAGTGGCTG 540
ATCTAGTGAG ATTCTAGAGC CGACAGTTAA AGTCTGGATG GGAGAAAGAA TGTTAATTAT 600
CGACAAAGAT AATGTAGCGT ATTTGTAAAA ATGTGTACAA ATAGGCTTAT TTAACGATAA 660
ATTTTTCTCC TTTGCATCTT AATTCATGAT GTGAGGATTT TTTGTTTATA GAGGTGATCA 720
TTTGAGTCAA TTTATGGATT ATGCGATTCA ACTTCCAAAT ATGGTACAAG GTCANACAGG 780
TGTTAATCCA CCCGTTGGCG CTGTTGTAGT TAATGAAGGT AGGATTGTTG GTATTGGTGC 840

ACACTTGAGA AAAGGTGACA AGCATGCGGA GGTTCAAGCA CTTGATATGG CACAACANAA 900
TGCTGAAGGT GCGACGATTT ATATTACGTT AGAGCCATGT AGTCATTTTG GTTCAACACC 960
ACCCTGTGTT AACAAAATTA TTGATTGTAA GATAGCANAA GTAGTATTAC NCAACANAAG 1020
ACAATTCCGT TAGACACACA TGGGTGATGA GACGTTACGG GGCTCCACGG TATTTGAGGG 1080
TTGAATTGCG TTGGATGATG AACGGGCATC ACAATTATAC CAAAGACTTT TTTTAAAGCA 1140
AAAAGCAAAG CAACTTGCCA CAAAATTACA GTGAAAGTNT CTTGAAAGTT TAGATGGGTA 1200
AACAAAGCGA ATTGATAATG GACAAAGTCA ATGGATTACT AACAAAGAGG TTAAACAAGA 1260
TGTCTATAG 1269

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 99 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asp Tyr Ala Ile Gln Leu Pro Asn Met Val Gln Gly Xaa Thr Gly
1 5 10 15
Val Asn Pro Pro Val Gly Ala Val Val Val Asn Glu Gly Arg Ile Val
20 25 30
Gly Ile Gly Ala His Leu Arg Lys Gly Asp Lys His Ala Glu Val Gln
35 40 45
Ala Leu Asp Met Ala Gln Xaa Asn Ala Glu Gly Ala Thr Ile Tyr Ile
50 55 60
Thr Leu Glu Pro Cys Ser His Phe Gly Ser Thr Pro Pro Cys Val Asn
65 70 75 80
Lys Ile Ile Asp Cys Lys Ile Ala Xaa Val Val Leu Xaa Asn Xaa Arg
85 90 95
Gln Phe Arg

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGAAGGTAG GATTGTTGGT A 21

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AGTCTTGGTA TAATTGTGAT GC 22

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3336 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGGATTATG CGATTCAACT TGCAAATATG GTACAAGGTC AAACAGGTGT TAATCCACCC 60
GTTGGCGCTG TTGTAGTTAA TGAAGGTAGG ATTGTTGGTA TTGGTGCACA CTTGAGAAAA 120
GGTGACAAGC ATGCGGAGGT TCAAGCACTT GATATGGCAC AACAAAATGC TGAAGGTGCG 180
ACGATTTATA TTACGTTAGA GCCATGTAGT CATTTTGGTT CAACACCACC CTGTGTTAAC 240
AAAATTATTG ATTGTAAGAT AGCAAAAGTA GTATACGCAA CAAAAGACAA TTCGTTAGAC 300
ACACATGGTG ATGAGACGTT ACGGGCTCAC GGTATTGAGG TTGAATGCGT TGATGATGAA 360
CGGGCATCAC AATTATACCA AGACTTTTTT AAAGCAAAAG CAAAGCAACT GCCACAAATT 420
ACAGTGAAAG TATCTGCAAG TTTAGATGGT AAACAAGCGA ATGATAATGG ACAAAGTCAA 480

TGGATTACTA ACAAAGAGGT TAAACAAGAT GTCTATAAGT TAAGACATCG ACACGACGCA 540
GTGTTAACTG GAAGACGTAC AGTTGAATTA GATGATCCAC AATATACTAC ACGTATTCAA 600
GATGGAAAAA ACCCTATAAA AGTAATATTG TCTAAGTCTG GGAATATTCA TTTTAATCAG 660
CAAATTTATC AAGATGAATC AACACCAATT TGGATATATA CTGAAAATCC AAATTTAACA 720
AGCAATCAAA CACATATTGA AATTATTTAC TTGAAGTCTT GTGATTTAAC AACAATTCTT 780
CACAATTTAT ATAAAAGAGG AGTTGGAACT TTGCTAGTCG AGGCAGGTCC AACCACTACT 840
TCAGAATTCT CCATCTATTA TATAGATGAA TTTATTCTCT ATTATGCCCC GAAATTAATT 900
GGCGGATCTG GAAATTATCA ATTTTATCAA ACAAATGATG TGATTGAGAT ACCAGATGCG 960
AACCAATTTG AAATTGTTCA TTCCGAGTTA TTAAATCAAA ATGTTAAATT AACTTTACGA 1020
AAGAAGTGAT GATGCATGTT TACTGGCATC GTTGAAGAAA TAGGTGTCGT TAAAAGTGTT 1080
CAAATTCGTC AATCAGTCAG GACGATTGAA ATCGAAGCAC ATAAGATTAC GGCAGATATG 1140
CATATTGGTG ATTCCATCAG TGTTAATGGT GCATGTTTAA CAGTGATTGA TTTTGATCAG 1200
ACATCTTTTA CTGTTCAAGT AATTAAAAGC ACTGAAAATA AAACCTATTT AGCAGATGTT 1260
AAGCGACAAT CAGAAGTAAA TTTAGAGCGT GCCATGAGTG GTAACGGTAG GTTTGGTGGA 1320
CATTTTGTCC TCGGTCATGT AGATGAACTA GGAACAGTTT CAAAAATAAA TGAAACAGCC 1380
AATGCCAAAA TTATTACGAT TCAATGTAGC CAACATATTA ATAATCAGTT AGTTAAGCAA 1440
GGGTCTATTA CTGTGGATGG TGTAAGTCTA ACGGTATTTG ATAAGCATGA TAACAGTTTT 1500
GACATTCATC TTATTCCAGA AACGAGGCGT TCAACGATTT TATCATCCAA AAAATTAGGA 1560
GATAAAGTAC ATTTAGAAAC AGACGTTTTG TTTAAATATG TTGAAAATAT TTTAAATAAA 1620
GATAAAGACC AATTATCTGT AGATAAATTA AGAGCATTTG GTTTTTAGGA GGGGTAGCAT 1680
GCAATTCGAT AATATTGACA GTGCTTTAAT GGCTTTAAAA AATGGAGAAA CAATTATTGT 1740
AGTAGATGAT GAGAATCGTG AAAATGAAGG TGATTTAGTA GCGGTTACTG AATGGATGAA 1800
CGATAATACC ATTAATTTTA TGGCGAAAGA AGCAAGGGGA TTAATATGCG CACCAGTGTC 1860
TAAAGATATT GCACAACGTT TGGATTTGGT ACAAATGGTT GATGATAACT CCGACATCTT 1920
TGGTACGCAA TTTACAGTGA GTATTGATCA TGTAGATACA ACAACAGGAA TTAGTGCTTA 1980
TGAACGTACA TTGACTGCCA AAAAGCTCAT TGATCCTAGT AGTGAAGCTA AAGATTTTAA 2040
TCGTCCTGGT CATTTATTTC CATTAGTAGC ACAAGATAAA GGCGTATTAG CTAGAAATGG 2100
ACACACAGAA GCGGCTGTTG ATTTAGCTAA ACTTACTGGT GCCAAGCCCG CTGGTGTCAT 2160
TTGTGAGATT ATGAATGATG ACGGCACGAT GGCGAAAGGA CAAGATTTAC AAAATTTTAA 2220
AGAAAAACAT CAATTAAAGA TGATTACGAT TGATGATTTA ATTGAATATC GTAAAAAATT 2280
AGAACCAGAA ATTGAATTTA AGGCAAAAGT GAAAATGCCT ACAGATTTCG GAACATTTGA 2340
TATGTATGGT TTTAAAGCGA CATACACAGA TGAAGAGATA GTTGTACTGA CAAAAGGTGC 2400
AATTCGACAA CATGAAAATG TACGCTTACA TTCTGCGTGC CTTACAGGCG ATATTTTCCA 2460
TAGTCAACGT TGTGATTGTG GTGCTCAACT TGAATCGTCT ATGAAGTATA TCAATGAACA 2520
TGGTGGCATG ATTATTTATC TACCTCAAGA AGGTCGTGGC ATAGGATTGT TAAACAAATT 2580
ACGCGCATAT GAATTAATTG AGCAAGGATA TGATACAGTA ACTGCAAATT TAGCATTAGG 2640
TTTTGATGAA GATTTACGAG ATTATCATAT TGCTGCACAG ATTTTAAAAT ATTTTAACAT 2700
CGAACATATC AATTTATTAA GTAATAATCC AAGTAAATTT GAGGGATTAA AACAATATGG 2760
CATTGATATT GCAGAAAGAA TTGAAGTTAT CGTACCAGAA ACGGTACATA ATCATGATTA 2820
TATGGTAACG AAAAAAATAA AAATGGGTCA TTTAATATAG GAGGACTTTA ACATGAATTT 2880

TGAAGGTAAA TTAATTGGAA AAGATTTGAA AGTTGCAATC GTAGTTAGTC GATTTAATGA 2940
TTTTATCACT GGAAGATTAC TTGAAGGTGC AAAAGATACT TTGATTCGAC ATGATGTTAA 3000
TGAAGACAAT ATTGATGTAG CATTTGTTCC TGGTGCGTTT GAAATTCCTT TAGTAGCTAA 3060
AAAATTAGCC TCATCAGGAA ATTATGATGC AATAATTACA TTAGGATGCG TAATTCGCGG 3120
TGCTACGTCT CATTATGATT ATGTTTGTAA TGAAGTGCGA AAGGTGTTTC TAAAGTAAAT 3180
GATCAAACTA ATGTACCAGT CATATTTGGT ATTTTAACGA CTGAAAGTAT TGAACAAGCT 3240
GTGGAAAGAG CAGGTACGAA AGCTGGTAAT AAAGGTGCCG AAGCAGCAGT AAGTGCAATT 3300
GAAATGGCTA ATTTATTAAA ATCTATAAAA GCATAG 3336

What is claimed is:

1. An isolated polynucleotide segment comprising: a first polynucleotide sequence, or the full complement of the entire length of the first polynucleotide sequence, wherein the first polynucleotide sequence is selected from the group consisting of:

(a) a polynucleotide consisting of SEQ ID NO:1; and,

(b) a nucleic acid sequence identical to the polynucleotide of (a) except that, over the entire length corresponding to the polynucleotide of (a), up to **thirty** nucleotides are substituted, deleted or inserted for every 100 nucleotides of the polynucleotide of (a).

2. The isolated polynucleotide segment of claim 1, wherein the first polynucleotide sequence is selected from the group consisting of: the polynucleotide of (a); and, a nucleic acid sequence identical to the polynucleotide of (a) except that, over the entire length corresponding to the polynucleotide of (a), up to **ten** nucleotides are substituted, deleted or inserted for every 100 nucleotides of the polynucleotide of (a).

3. The isolated polynucleotide segment of claim 1, wherein the first polynucleotide sequence is selected from the group consisting of: the polynucleotide of (a); and, a nucleic acid sequence identical to the polynucleotide of (a) except that, over the entire length corresponding to the polynucleotide of (a), up to **five** nucleotides are substituted, deleted or inserted for every 100 nucleotides of the polynucleotide of (a).

4. A vector comprising the isolated polynucleotide segment of Claim 1.

5. An isolated host cell comprising the vector of claim 4.

6. An isolated polynucleotide segment, comprising a first polynucleotide sequence, or the full complement of the entire length of the first polynucleotide sequence, wherein the first polynucleotide sequence is selected from the group consisting of

(a) a polynucleotide which encodes the same mature polypeptide, expressed by the ribG gene expressed by a polynucleotide comprising SEQ ID NO:1 contained in *Staphylococcus aureus* WCUH 29 contained in NCIMB Deposit No. 40771; and,

(b) a nucleic acid sequence identical to the polynucleotide of (a) except that, over the entire length corresponding to the polynucleotide of (a), up to **five** nucleotides are substituted, deleted or inserted for every 100 nucleotides of the polynucleotide of (a).

7. The isolated polynucleotide segment of claim 6, wherein the first polynucleotide sequence is selected from the group consisting of: the polynucleotide of (a); and, a nucleic acid sequence identical to the polynucleotide of (a) except that, over the entire length corresponding to the polynucleotide of (a), up to **three** nucleotides are substituted, deleted or inserted for every 100 nucleotides of the polynucleotide of (a).

8. An isolated polynucleotide segment, comprising a first polynucleotide sequence or the full complement of the entire length of the first polynucleotide sequence, wherein the first polynucleotide sequence hybridizes to the full complement of SEQ ID NO:1, wherein the hybridization conditions include incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml denatured, sheared salmon sperm DNA, followed by washing in 0.1x SSC at about 65°C.

9. The isolated polynucleotide segment of claim 8, wherein the first polynucleotide sequence is identical to SEQ ID NO:1 except that, over the entire length corresponding to SEQ

ID NO:1, up to **five** nucleotide are substituted, deleted or inserted for every 100 nucleotides of SEQ ID NO: 1.

10. The isolated polynucleotide segment of claim 8, wherein the first polynucleotide sequence is identical to SEQ ID NO:1 except that, over the entire length corresponding to SEQ ID NO:1, up to **three** nucleotide are substituted, deleted or inserted for every 100 nucleotides of SEQ ID NO: 1.

11. An isolated polynucleotide segment, comprising a first polynucleotide sequence or the full complement of the entire length of the first polynucleotide sequence, wherein the first polynucleotide sequence is selected from the group consisting of:

(a) a polynucleotide which encodes a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2; and,

(b) a nucleic acid sequence identical to the polynucleotide of (a) except that, over the entire length corresponding to the polynucleotide of (a), up to **five** nucleotides are substituted, deleted or inserted for every 100 nucleotides of the polynucleotide of (a).

12. A vector comprising the isolated polynucleotide segment of Claim 11.

13. An isolated host cell comprising the vector of claim 12.

14. The isolated polynucleotide segment of claim 11, wherein the first polynucleotide sequence is selected from the group consisting of:

(a) a polynucleotide which encodes a polypeptide consisting of the amino acid sequence set forth in SEQ ID NO:2; and,

(b) a nucleic acid sequence identical to the polynucleotide of (a) except that, over the entire length corresponding to the polynucleotide of (a), up to **five** nucleotides are substituted, deleted or inserted for every 100 nucleotides of the polynucleotide of (a).

15. A vector comprising the isolated polynucleotide segment of claim 14.

16. An isolated host cell comprising the vector of claim 15.

17. A composition comprising the isolated polynucleotide segment of claim 1, which isolated polynucleotide segment is according to the formula:

$$X\text{-}(R_1)_m\text{-}(R_2)\text{-}(R_3)_n\text{-}Y$$

wherein, at the 5' end of the molecule, X is hydrogen, and at the 3' end of the molecule, Y is hydrogen or a metal, R_1 and R_3 are any nucleic acid residue, m is an integer between 1 and 3000 or zero, n is an integer between 1 and 3000 or zero, and R_2 is the first polynucleotide sequence.

18. An isolated polynucleotide segment comprising: a first polynucleotide sequence, or the full complement of the entire length of the first polynucleotide sequence, wherein the first polynucleotide sequence is selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO:4;

(b) a polynucleotide consisting of SEQ ID NO:3; and,

(c) a nucleic acid sequence identical to the polynucleotide of (b) except that, over the entire length corresponding to the polynucleotide of (b), up to **thirty** nucleotides are substituted, deleted or inserted for every 100 nucleotides of the polynucleotide of (b).

19. The isolated polynucleotide segment of claim 18, wherein the first polynucleotide sequence is selected from the group consisting of: the polynucleotide of (b); and, a nucleic acid sequence identical to the polynucleotide of (b) except that, over the entire length corresponding to the polynucleotide of (b), up to **ten** nucleotides are substituted, deleted or inserted for every 100 nucleotides of the polynucleotide of (b).

20. The isolated polynucleotide segment of claim 18, wherein the first polynucleotide sequence is selected from the group consisting of: the polynucleotide of (b); and, a nucleic acid sequence identical to the polynucleotide of (b) except that, over the entire length corresponding to the polynucleotide of (b), up to **five** nucleotides are substituted, deleted or inserted for every 100 nucleotides of the polynucleotide of (b).

ABSTRACT OF THE DISCLOSURE

The invention provides ribG polypeptides and polynucleotides encoding ribG polypeptides and methods for producing such polypeptides by recombinant techniques. Also provided are methods for utilizing ribG polypeptides to screen for antibacterial compounds.

Docket No.: P50444-9

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

"ribG"

the specification of which (check one)
[X] is attached hereto.
[ ] was filed on as Serial No.
and was amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or Inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Number	Country	Filing Date	Priority Claimed

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

Application Number	Filing Date
60/011,888	20 February 1996

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

Serial No.	Filing Date	Status
PCT/US97/02318	19 February 1997	

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST-CLASS MAIL IN AN ENVELOPE ADDRESSED TO: ASSISTANT COMMISSIONER OF PATENTS, WASHINGTON, D.C. 20231, ON ______________________

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